Title: Evaluating the effect of age and area of residence in the metals and metalloids content in human hair and urban topsoils.

Author names and affiliations: Peña-Fernández A. [a,b], González-Muñoz M.J. [b], Lobo-Bedmar M.C. [c]

[a] School of Allied Health Sciences, De Montfort University, The Gateway, Leicester LE1 9BH, UK.

[b] Universidad de Alcalá, Unidad de Toxicología, Departamento de Ciencias Biomédicas, Crta. Madrid-Barcelona Km, 33.6, 28871 Alcalá de Henares, Madrid, Spain.

[c] IMIDRA. Departamento de Investigación Agroambiental. Finca el Encín, Crta. Madrid-Barcelona Km, 38.2, 28800 Alcalá de Henares, Madrid, Spain.

Corresponding author: Peña-Fernández Antonio

School of Allied Health Sciences, De Montfort University, The Gateway, Leicester LE1 9BH, UK.

Email: Antonio.Pena-Fernandez@dmu.ac.uk

Telephone: +44 116 201 3859

Abstract

Monitoring the levels of trace elements in hair can allow estimating the effects of the geographical location and also can provide a notion of the metal body burden. However, the use of human hair is controversial due to different confounding factors that could affect the presence of trace elements in hair. As a result, a comprehensive monitoring study was performed in Alcalá de Henares, one of the major cities in the Madrid Region, Spain. Trace elements have been monitored in urban topsoils and in human hair of two well-defined and healthy groups of population: children (6-9 years) and adolescents (13-16 years). The city was divided into four areas or zones with different characteristics to assess the possible effect of the area of residence and the age in the presence of Al, As, Be, Cd, Cr, Cu, Hg, Mn, Pb, Sn, Ti, Tl and Zn in soils and hair. There is no current hypothesis that explains the possible effect of the area of monitoring in the distribution of Be, Cr, Ni, Sn and Ti found in these urban soils, maybe because urban soils receive high disturbance and there are many factors involved. The presence of most of the trace elements monitored was significantly higher in the hair of the children population, except for Sn and Zn. This could be attributed mainly to dietary habits. Other factors influencing metal content in hair such as environmental factors would have had a minimal effect in the population groups here studied. Finally, none of the levels of trace elements studied in hair were significantly correlated with levels measured in the topsoils of public parks in Alcalá de Henares, with the exception of Pb in adolescent participants.

Keywords:

Biomonitoring, metals and metalloids, human hair, soils, age, area of residence.

1. INTRODUCTION

The human population boom and rapid urbanization and industrialization have produced a serious problem of contamination by metals and metalloids (trace elements) in urban environments. This is a cause for concern due to their battery of deleterious effects even at long-term low-dose levels of exposure (Peña-Fernández et al., 2014a). Owing to the accumulative capacity of metals and metalloids in ecosystems and in humans, environmental and biomonitoring programmes are robust, efficient and practical tools to protect the public health, especially in urban environments in which these substances are progressively increasing (Peña-Fernández et al., 2014b). These tools are also particularly useful to protect the health of children who are particularly vulnerable to environmental contaminants (Molina-Villalba et al., 2015). The tools can help to inform decisions on whether the urban environment requires recovery and restoration.

Infants and children are more susceptible to environmental pollutants than adults as they have a less developed blood-brain barrier (Jarup, 2003), they breathe more air, drink more water and eat more food per unit weight than adults (Moya et al., 2004). Moreover, infant and children are more likely to be affected by contaminated soils than adults due to their behavioural patterns and higher absorption rates from the gastrointestinal tract (Johnson and Bretsch, 2002). Thus, Callan et al. (2012) have reported cognitive and neurobehavioral changes in children exposed to concentration of trace elements below of thresholds considered safe. As a result, children and young people should be a target population group in environmental and public health studies.

Human hair is a useful tissue sample for non-invasive environmental health surveys and is considered a good matrix for estimating environmental exposures (Varrica et al., 2014). Blood and urine are the most widely accepted tissue samples for biomonitoring metals and metalloids exposure. However, hair is stable, and for the purposes of sampling, it is both easily accessible and more acceptable to the target group than collecting blood. Moreover, human hair better reflects long-term exposure than blood, as human hair grows approximately 10 mm per month, providing an average of the growth period (Gil et al., 2011).

The use of hair as a biomonitor can be controversial, as hair has several limitations that have been discussed comprehensively in Peña-Fernández et al. (2014a) and Molina-Villalba et al. (2015). Nevertheless, the use of the methodology and strict inclusion criteria described in Peña-Fernández et al. (2014a) might facilitate the use of human hair as a screening tool in environmental bio-monitoring studies by reducing or minimising some of the factors that influencing the presence of trace elements in this tissue.

In Spain, few biomonitoring studies have involved infants and children. Furthermore, environmental monitoring studies in urban areas have recently grown in importance to protect their citizens (Peña-Fernández et al., 2014a), although these studies are almost non-existent in the Madrid Region, despite it being one of the most populated regions in Spain and continuing to expand through urban development.

As a consequence, a comprehensive environmental and human monitoring study was undertaken in Alcalá de Henares, as it is one of the largest cities in the Madrid Region, Spain. Metals and metalloids were monitored in topsoils from Alcalá de Henares' urban parks, as urban soils can be a significant source of trace elements for children, and act as tracers of environmental pollution and as "health indicators" (Massas et al., 2009). Moreover, metals and metalloids were monitored in hair of two well-defined and healthy Spanish groups of the population living in Alcalá de Henares: children (6-9 years) and adolescents (13-16 years). Soil and hair samples were collected from different zones or areas of residence. The levels of metals and metalloids in soils and in children's and adolescents' hair samples have been discussed in previous papers (Peña-Fernández et al., 2014a, 2014b, 2016). This article focus on the evaluation of the possible effect of the age and the area of residence in the presence of metals and metalloids in human hair and urban topsoils in order to have a better understanding of hair as a suitable and reliable biomarker. The methods employed for selecting participants have shown to reduce factors that influence the presence of trace elements in human hair (Peña-Fernández et al., 2014a). Moreover, monitoring studies of trace elements on a well-defined population can be used to estimate the effects of the geographical location on the public health of their citizens (Avino et al., 2013), and could provide robustness to a risk assessment study.

For this study, levels of aluminium, arsenic, beryllium, cadmium, chromium, copper, mercury, manganese, lead, tin, titanium, thallium and zinc were monitored in topsoils from urban parks in Alcalá de Henares, Spain, and in hair collected from children and adolescents living in this city, and their concentrations compared.

2. MATERIAL AND METHODS

2.1. Study design and recruitment

Alcalá de Henares, Spain, is a UN World Heritage city (latitude: 40° 28′ 49″ N — longitude: 3° 22′ 9″). It is 35 km from Madrid city and 15 km from the international airport of Madrid–Barajas. It has a population of about 200,000 inhabitants in an area of 88 km^2, making it one of the most populated cities of the Madrid Region. There is a great deal of industrial activity in Alcalá de Henares and also a high traffic density.

In order to study the possible effect of the area of residence, the city was divided into 4 zones (Figure 1): zone I had a higher density of green areas and open spaces; zone II was a more urban environment, with a higher number of buildings; zone III had a higher density of traffic; zone IV is home to industrial activities.

A total of 97 topsoil samples (0–3 cm depth) were randomly sampled from different parks and recreation areas in Alcalá de Henares in July 2001 as follows: 25 soil samples were taken in each of zone I and IV, 23 soil samples in zone II and 24 soil samples in zone III. Soil samples were dried at room temperature for 2 weeks, ground and sieved with a 2 mm sieve to remove stones, coarse materials, and other debris (Schuhmacher et al., 1996).

Hair samples were collected between April and May of 2001 from 117 healthy Spanish children aged 6-9 years (47 boys and 70 girls) and 96 healthy adolescents aged 13-16 years (28 boys and 68 girls). The candidates were selected were all of Caucasian ethnicity and had been permanent residents of Alcalá de Henares; all met the strict inclusion criteria described in Peña-Fernández et al. (2014a) after monitoring all the schools (private and public) in Alcalá de Henares. The numbers in these two groups were large enough to be representative of Alcalá's young population. Tables 1 and 2 show the total numbers of children and adolescents that participated in each of the residences or zones into which Alcalá de Henares was divided. A brief description of the methods used for selecting participants is as follows: written agreement was obtained from the parents or legal guardians (after a face-to-face meeting) as well as from the schools' directors; a lifestyle questionnaire was performed to obtain information regarding sex, age and life-style habits for all participants; participation was restricted to the healthy Spanish Caucasian young population who did not use any hair treatments (stains, fixers, permanents waves, etc.) given the influence of these factors on the utility of hair as a biomonitor (Peña-Fernández et al., 2014a); only those who have been living in Alcalá de Henares continuously since birth were selected. In addition, candidates were excluded if they were prescribed medical treatments for long-term health conditions or had orthodontic treatment. The study followed the guidelines of the Helsinki Declaration.

Hair samples 1-2 cm long were cut with stainless steel scissors from the nape of the neck, close to the occipital region of the scalp. The methodology and selection criteria described here could be the basis for further environmental studies to identify environmental contamination in urban settlements that will threat the human health.

2.2. Soil analysis

To estimate the geochemical distribution and significance of trace elements in topsoils from Alcalá's urban parks and recreation areas as a potential source of trace elements, total concentration of Al, As, Be, Cd, Cr, Cu, Hg, Mn, Pb, Sn, Ti, Tl and Zn were monitored in each sample collected in each zone. All topsoils and hair samples were collected during the spring and summer of 2001. Total concentrations of trace elements are comprehensively described in Peña-Fernández et al. (2014b) but can be found in Table 3 for information purposes.

Physicochemical characteristics of the soils were also determined per zone of sampling as these parameters could play a role in the possible relationships between trace elements and their presence in soils (Vanek et al., 2010). The pH, electrical conductivity (E.C.), organic matter content (O.M.) and the texture (percentage of sand, clay and silt) of these soils were determined according to standard methods (MAPA, 1994; Brady and Weil, 2001; FAO, 2009).

2.3. Hair analysis

The concentrations of the same trace elements (Al, As, Be, Cd, Cr, Cu, Hg, Mn, Pb, Sn, Ti, Tl and Zn) were also monitored in all the hair samples by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES, Thermo Jarrel Ash ICAP 61), and are described for children and adolescents in Peña-Fernández et al. (2014a) and Peña-Fernández et al. (2016), respectively.

2.4. Statistical analysis

The data of the following trace elements were logarithmically transformed to normalizing distributions: Cu and Pb in soil samples; Al, Cu, Hg, Mn, Ni and Pb in children's hair; and Cu, Hg and Mn in the adolescents' hair. Generally, when the observations have a high variability in results, it is accepted the standardization of the results as a previous step to the statistical analysis (Xu and Tao, 2004).

Statistical significance of the data was computed by one-way analysis of variance (ANOVA). The Kolmogorov–Smirnov test was used to confirm that the values were normally distributed, while homogeneity of the variances was assessed using Snedecor's F-test. In addition, the Fisher's least significant difference (LSD) test was used to determine which means differed significantly from the others using a significance level of 0.05 or less. All calculations were performed using the statistical package SPSS 22.

2. RESULTS

2.1. Soil samples

The physicochemical characteristics of the Alcalá de Henares's soils are provided in Table 4. The soils studied are moderately basic (pH values range between 7.65 and 7.97). The content of organic matter varied very little between areas, with percentages ranging from 1.22% to 1.36%. The pH and electric conductivity values were similar or lower to those described in other Spanish urban soils such as in San Martin de la Vega, Madrid (Chicharro et al., 1998), Sevilla (Madrid et al., 2004) and Tarragona (Schuhmacher et al., 2003). However, the organic matter content was lower in Alcalá's soils than those provided in those studies. A high content of organic matter would be expected in the upper layer of urban soils as these soils are normally covered with grass but most of the samples were collected in areas with no herbaceous cover. Alcalá's soils are classified as sandy loam according to the standard methodology (Brady and Weil, 2001; FAO, 2009). This texture has been described as common in soils from public parks and recreational areas.

Metal and metalloid concentrations in topsoils samples are illustrated in Figure 2 according to the zone in which was divided the urban area of Alcalá de Henares. Be ($p<0.001$), Cr ($p<0.05$), Ni ($p<0.01$), Sn ($p<0.01$) and Ti ($p<0.05$) have shown statistical significance different between zones. All of these elements have shown a different behaviour in the soil samples per zone studied. Thus, zone I was significantly contaminated by Be, Cr and Ti; zone III presented significant higher levels of Cr, Sn and Ti; and zones II and IV presented the smaller levels of those metals that shown a dependency with the area of residence. This result was unexpected as the zone I had a higher density of green areas, and therefore a lower density of traffic and industries than the other zones (Figure 1). Zone I was expected to be, *a priori*, one of the least polluted.

A statistical correlation study between the variables analysed in soils (pollutants and soil characteristics) was also performed to detect any possible relationships between them. This study is reported in two tables (Tables 5 and 6) due to the large number of data although all the variables and samples were considered in conjunction. The correlation study showed that Al, As, Be, Cd, Cr, Mn, Ti and V were positively and significantly correlated with each other, although the correlation coefficients were not very high (Table 5). Cd, Cu, Pb and Zn have also shown a positive and significant correlation, indicating a strong relationship between them, as it is shown in Table 5. For the rest of variables determined in these soils, Be, Cu, Pb and Sn have shown a relationship with different physicochemical properties as follows: Be and E.C. ($r = 0.704$; $p <0.05$); Cu and percentage of silt ($r = 0.720$; $p <0.01$) and sand ($r = -0.646$; $p <0.05$); Pb and E.C. ($r = 0.949$; $p <0.001$); and Sn and E.C. ($r = 0.902$; $p <0.001$) (Table 6).

2.2. Hair samples

Figures 3 and 4 illustrate the metal and metalloid concentrations in children's and adolescents' hair collected in each zone in Alcalá de Henares, respectively. No children were monitored in zone III due to the lack of schools in this zone, as described in Table 1. In the case of children, Cr ($p<0.001$) and Hg ($p<0.01$) were the only elements significantly affected by the area of residence (Figure 3). The metal content in the adolescents' hair samples monitored showed significant differences between zones of residence for Cr ($p<0.01$), Cu ($p<0.05$), Hg ($p<0.05$), Pb ($p<0.01$) and Sn ($p<0.05$) (Figure 4). The concentrations of trace elements and significance levels for the elements significantly affected by the area of residence are also presented in Tables 7 and 8 for children and adolescents participants, respectively. For both groups of population, the metal content in hair revealed a dependency with the zone of residence only for Cr and Hg, although these two metals have shown a different behaviour in each group monitored. Thus, while Cr level in children's hair was significantly higher in zone IV, this was significantly lower in the adolescents' hair analysed. Meanwhile, although Hg was significantly higher in the hair of both groups monitored in the zone IV, the levels of this metal shown an opposite tendency in hairs collected in the zone II between both groups.

The possible influence of age on the presence of trace elements in the hair at young stages was also evaluated (Table 9). All trace elements monitored in Alcalá de Henares's hair have shown significant differences between both groups, with the exception of Ti, as follows: Al ($p<0.01$), Cd ($p<0.001$), Cr ($p<0.001$), Cu ($p<0.05$), Hg ($p<0.001$), Mn ($p<0.001$), Pb ($p<0.001$), Sn ($p<0.01$) and Zn ($p<0.001$). All the trace elements were significantly higher in the child population but for Sn: 1.29 vs. 1.52 µg/g, and for Zn: 85.58 vs 148.25 µg/g (Table 9).

2.3. Soils and hair samples

It was thought appropriate to conduct a statistical correlation study between those pollutants that have shown a dependency with the area or zone of sampling in soils, i.e. Be, Cr, Sn and Ti, with their presence in hair by area of residence, and for both groups of population.

None of the levels of trace elements studied in hair were significantly correlated with levels measured in topsoils of public parks in Alcalá de Henares, with the exception of Pb in adolescent participants living in zone II ($r = 0.483$; $p = 0.050$).

4. DISCUSSION

4.1. Effect of the area of sampling in the levels of trace elements in **soils** collected in Alcalá de Henares

The differences observed in the presence of trace elements due to the area could be attributed to a multitude of factors, which are described below.

The background level of metal and metalloid elements in any soil is strongly associated with the geology of the area. According to local geological studies, Alcalá de Henares is situated in the central regions of the Tajo river pit, filled with Mesozoic, Paleogene and Neogene sediments. The Neogene sediments have carved its relief, basically quaternary (Acaso et al., 2007), a situation that may have contributed to the natural presence of different trace elements in a high concentration when compared with other urban areas, as suggested previously for As in these soils (Peña-Fernández et al., 2014b). This would be expected to have some influence on the presence of these elements found between areas due to their closeness.

However, the rate and influence of weather (mainly wind direction and strength, and rainfall), and the amount and variability of different anthropogenic activities that emit pollutants (mainly industries, heating and traffic), are not homogeneous in time and could affect the presence of trace elements in topsoils. This will cause what is called “diffuse pollution” (Tume et al., 2008), an effect that means that the presence of contaminants in the soil does not follow the expected pattern.

Therefore, the levels of pollutants here monitored would be a reflection of several sources of these pollutants at the same time, both natural (geological, hydrological, meteorological) and anthropogenic (Aelion et al., 2009; Mahanta and Bhattacharyya, 2011), many of which are today unknown or are not well-described.

In addition, the concentration of trace elements in soils can show high variability even at small distances, especially in urban environments (Dao et al, 2010). Thus, identification of both natural and anthropogenic emission source(s), as well as the possible justification of the presence of a given metal in this type of soils, involves a high degree of complexity, based on the inherent characteristics that define these types of soils. Urban soils receive high disturbance due to industrial and urbanization activities, traffic, type of soil and climate of the area, population growth, etc. (Rimmer et al., 2006). These events occur more significantly in parks and public gardens where soil is regularly distorted by irrigation, fertilizer deposition, application of pesticides, elimination of ornamental plants, trampling of visitors, etc., factors that will make this type of study extremely complex (Rimmer et al., 2006; Wong et al., 2006). Thus, although the characteristics and physicochemical properties of the soil could affect the presence of trace elements in soils, our results have not shown any strong and significant relationship between them and their level of contamination (Table 6). However, this might be due to the very little variability observed in the different soils' characteristics determined between zones (Table 4), mainly due to their proximity, although a better understanding of the degree of interaction of trace elements with the different constituents of the soil would be needed.

Currently, very little is known about the fluctuations of trace elements in urban soils between near zones even though there is a clear distinction between them, and there is no hypothesis in the literature that explains and/or justifies the fluctuations here observed. Moreover, hypotheses proposed so far that could explain partially the variability of these substances in topsoils are disconnected and not well defined. For instance, despite the presence of Pb in soils generally being linked to traffic density, some authors have found higher concentrations of this metal in areas with relatively limited traffic compared to those with a high density, as Ruiz-Cortés et al. (2005) found in topsoils in Sevilla, Spain. The concentrations of Pb in Alcalá's soils have not shown zone-dependence although previous monitoring samples performed in the same area in a different year have shown higher levels of this metal in zones I and III ($p<0.05$; data not published; Peña-Fernández, 2011). However, this could be partially attributed to its well-described wide environmental distribution (Madrid et al., 2006; Zhang, 2006).

Furthermore, Dao et al. (2010) have pointed out that the high variation in the metal content found in soils in small urban areas makes it very difficult to perform a monitoring sampling appropriate for geochemical studies. New research techniques and methods would be necessary to study the effect of the area in the presence of trace elements in urban topsoils (Wong et al., 2006).

4.2. Effect of the area of residence in the levels of trace elements in **children's and adolescents' hair** in Alcalá.

Only Cr and Hg have exhibited an area of residence dependency in both groups monitored, although they have shown a different behaviour as reported previously (Figures 3 and 4). It is known that the presence of trace elements in hair could reflect local environmental conditions and a geographic area has a typical profile of hair metal composition of its habitants (Tamburo et al., 2015). We have observed concentration trends in different trace elements determined in hair of two well-defined groups of population in function of the area of residence. Moreover, levels of Hg in Alcalá's soils were lower than the limit of detection. This might indicate that the presence of trace elements in hair of the participants would be mainly affected by nutritional and socioeconomic factors (Granero et al., 1998; Özden et al., 2007), rather than environmental factors. However, the proximity of the different areas and the current lack of knowledge about the toxicokinetics of excretion of these contaminants through hair make this study very complex. Thus, the dependence of the area of residence observed in the hair presence of Cr and Hg found in both Alcalá's groups could be attributed to the nutritional habits since this has been described as the major source of exposure of these two metals following occupational exposure (Granero et al., 1998; Storelli, 2009; Wranová et al., 2009). Díez et al. (2009) have pointed out that the Spanish preschool population is widely exposed to inorganic and organic Hg forms through food intake. However, more studies are needed as we have found that the hair would not be a good biological indicator of exposure to trace elements through the diet (González-Muñoz et al., 2008).

The effect of the dietary habits and/or socioeconomic factors could have also played an important role in the presence of Cu, Pb and Sn in adolescents' hair, as these metals have shown significant differences between areas of residence (Table 8). However, information about dietary habits and socioeconomic factors were not collected in detail so this is a limitation of this study. A comprehensive correlation study performed between trace element average composition in Alcalá de Henares' hair and the reference doses of the same species in the main environmental pollution sources (Sabbioni et al., 1981; Avino et al., 2013) have shown a very good correlation between elements and food (R^2=0.942) and water (R^2=0.876) suggesting that the monitored groups were mainly exposed to the analysed metals and metalloids through their diet (more information about this correlation study is described in Peña-Fernández et al., 2016). Furthermore, none of these trace elements have been correlated with their presence in urban soils as highlighted above, a fact that might corroborate the above hypothesis, *i.e.* Alcalá de Henares' environment would have not been a significant factor in the presence of trace elements in the human hair monitored.

Despite the difficulty and complexity of this type of studies, the analysis of the influence of the geographic area in the metal hair content in monitoring studies can be really important and should be considered in environmental studies, as this could be of public health relevance. Thus, for instance, children that live in zones II and IV of this study have presented levels of Hg in hair that exceed the threshold level of 1 μg/g above which cognitive and neurological damage has been described (Freire et al., 2010), a risk that is not seen in hair from schoolchildren living in zone I (Figure 3). The possible source(s) of Hg in the Alcalá de Henares's child population should be carefully studied to take the necessary preventive and corrective measures to protect the public's health against this neurotoxic metal. Particularly, the intake of fish and seafood in this group should be analysed as it has been described as the main source of Hg for humans, as shown in other studies (Castaño et al., 2015).

4.3. Effect of the **age** on trace elements in human hair

All the trace elements monitored have shown significant higher concentrations in hair collected in the children participants aged 6-9 years than in adolescents aged 13-16 years, except for Sn: 1.29 vs. 1.52 μg/g, and for Zn: 85.58 vs 148.25 μg/g (Table 9). In general, despite scientific evidence that the presence of metal and metalloids increases in hair with time (Amaral et al., 2008), numerous studies have shown that the levels of these substances were higher in the younger portion of the general population (Sanna et al., 2003). Thus, Kordas et al. (2010) have observed that the presence of As, Cd and Pb were higher in the hair of infants 6-37 months than in the hair of their respective mothers. This finding would be in agreement with our results. The increased presence of these substances in the earliest ages could be attributed to the physiological characteristics of this group, as previously commented, higher absorption rates as children breathe more air, drink more water and eat more food per unit weight than adults, and higher absorption rates from the gastrointestinal tract.

Moreover, children will be easily exposed to environmental contaminants due to their behavioural patterns (Molina-Villalba et al., 2015).

The levels of Al in the hair of the adolescent population (5.34 µg/g) were significantly lower ($p<0.01$) to those found in children (9.05 µg/g) (Table 9). The decrease in the presence of this toxic metal in hair could be attributed to the immaturity of the urinary system of infants and children, as this organ system is the major route of excretion of Al. As a consequence of the zero or low detoxification rate of Al, this metal would be accumulated in the children's body (Bouglé et al., 1997), and its presence would be increased in tissues such as hair. Our results are in agreement with those described in other studies (Paschal et al., 1989; Yasuda et al., 2009).

Regarding the presence of Cd in this matrix, and despite the low number of samples in which it was detected, this was significantly higher in Alcalá's children (0.52 vs. 0.11 µg/g; $p<0.001$; Table 7). This would be consistent with other studies that have reported higher levels of Cd in the hair in younger ages (Lekouch et al., 1999; Bosque et al., 1991). Dietary factors would play a significant role in the presence of Cd in the hair of the groups monitored, as only non-smokers were selected for this study (as described in the strict inclusion criteria; Peña-Fernández et al. 2014a). Jarup and Akesson (2009) have reported that the major source of exposure to Cd in non-smokers is diet.

The presence of Cr and Cu were also significantly higher in the hair of the child participants (Table 9). This would be consistent with the results described by Perrone et al. (1996), which determined that the levels of these metals in hair increase from birth to eight years, and then decline.

The range of total Hg determined in the adolescent population of Alcalá de Henares (0.09 to 2.41 µg/g) was significantly lower ($p<0.001$) than that monitored in children (0.16 to 4.86 µg/g). Pesch et al. (2002) have reported that the levels of total Hg would correlate negatively with age, in a study conducted on 245 children aged 8-10 years. However, Budtz-Jørgensen et al. (2004) have observed an opposite dependence with age, *i.e.* the presence of total Hg would increase with age in this matrix. The elevated presence of this particular neurotoxin in children could be attributed to the physiological characteristics of this group described above, so children would be more exposed to Hg through dietary sources. In addition, due to the immaturity anatomic-functional typical of the paediatric age, the capacity of detoxification and excretion of contaminants is different in children than in adults (Landrigan et al., 2010).

With respect to Mn, the average concentration in the hair of the adolescents was significantly lower than in children (0.14 vs. 0.30 µg/g; $p<0.001$; Table 9). Children are especially sensitive to the toxicity of this metal, as infants accumulate more Mn than adults due to their greater absorption rate of this element (Gerber et al., 2002). However, Bouchard et al. (2007) found an opposite trend with age, ie the presence of Mn was positively correlated with age, although the authors did not consider different factors which could affect the presence of trace elements in the hair, such as the length of residence in the community.

Contrary to the other trace elements, the levels of Sn and Zn were significantly higher in the hair of the adolescent group (1.52 vs. 1.29 µg/g; $p<0.01$) and (148.25 vs. 85.58 µg/g; $p<0.001$), respectively (Table 9). Ti, for its part, did not show dependency with the age (Table 9). The trend observed in the levels of Zn would be consistent with other studies that have reported that its concentration increases gradually until age 20 years, irrespective of sex (Sakai et al., 2000). The authors have attributed this to the fact that growth processes require higher amounts of this essential element, especially in the period of adolescence. Sn and Ti are metals that have been monitored very little although the differences found here for Sn might be attributed to similar causes as those described for the other trace elements, ie the physiological characteristics of children, although they are not well-understood.

5. CONCLUSIONS

There is no current hypothesis that explains the significant fluctuations in the trace element content observed in urban soils collected in the above adjacent areas, as there are many factors involved. However, a better knowledge in the effect of the soil characteristics and area monitored is critical due to their potential implications for human health. Thus, the concentration of some highly toxic elements such as Hg can dramatically vary between zones even at small distances.

In general, Alcalá de Henares' child population (6-9 years) have presented significantly higher levels in hair for the entirety of trace elements monitored than the adolescents' counterparts (13-16 years), except for Sn and Zn. This could be attributed mainly to dietary habits, although a comprehensive study of the possible role of diet in the excretion of metals and metalloids in hair in these groups is needed. Other factors influencing metal content in hair such as environmental factors would have had a minimal effect in the population groups studied here. This hypothesis is based in the lack of correlation observed between hair and soil samples for the trace elements determined and the fact that we monitored two well-defined and healthy groups during the same period of time and with the same methods for minimising the effect of confounding factors that can influence the presence of trace elements in human hair.

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Table 1. Number of **children** (N) (6-9 years) that have participated in each zone in Alcalá de Henares.

Zone	School	N	Boys	Girls
I	1	21	7	14
II	1	27	11	16
	2	9	4	5
	3	24	10	14
IV	1	36	15	21

Table 2. Number of **adolescents** (N) (13-16 years) that have participated in each zone in Alcalá de Henares.

Zone	School	N	Male	Female
I	1	10	3	7
	2	26	3	23
II	1	3	2	1
	2	11	6	5
	3	6	1	5
III	1	24	5	19
IV	1	15	8	7
	2	2	1	1

Table 3. Statistical summary of metals and metalloids in **urban soils** of Alcalá (mg kg^{-1}) (data collected from Peña-Fernández et al., 2014b).

Element	LoD	N	Arithmetic mean	Geometric mean	Range
Al	2.0	97	5,797 ± 2,646	5,135.27	762.02-12,672
As	0.01	96	4.83 ± 2.10	4.45	1.87-11.68
Be	0.01	97	0.75 ± 0.50	0.62	0.17-2.57
Cd	0.002	91	0.11 ± 0.06	0.10	0.03-0.33
Cr	0.004	89	8.37 ± 3.67	7.41	1.32-16.45
Cu	0.004	95	10.78 ± 6.44	8.99	2.21-38.08
Hg	0.002	0	ND	ND	<0.002
Mn	0.2	95	99.27 ± 40.09	90.15	17.91-188.17
Ni	0.2	97	6.56 ± 0.49	6.54	4.49-7.15
Pb	0.002	96	41.32 ± 47.59	26.24	3.03-290.46
Sn	0.01	93	0.31 ± 0.08	0.30	0.16-0.58
Ti	0.02	95	77.91 ± 45.34	66.27	15.31-234.93
Tl	0.002	97	0.12 ± 0.05	0.11	0.03-0.25
V	0.004	96	9.05 ± 4.04	8.10	1.65-18.29
Zn	0.02	90	34.51 ± 16.50	29.94	5.81-78.67

LoD = limit of detection (mg kg^{-1}); N = number of samples above LoD; Arithmetic mean results are presented as mean values ± S.D.; ND = Not detected.

Table 4. Physicochemical characteristics of Alcalá de Henares' soils for zones

Zone	pH	E.C. (dS/m)	O.M. (%)	Sand (%)	Clay (%)	Silt (%)
I	7.68 ± 0.22[a]	697.4 ± 476.9[ab]	1.22 ± 0.70[a]	40,35[a]	2,42[a]	37,32[ab]
II	7.65 ± 0.26[a]	834.9 ± 324.6[b]	1.22 ± 0.43[a]	31,74[b]	7,88[ab]	44,09[ab]
III	7.97 ± 0.33[a]	540.0 ± 343.1[a]	1.36 ± 0.50[a]	34,01[b]	16,30[c]	41,48[ab]
IV	7.68 ± 0.47[a]	521.8 ± 222.4[a]	2.86 ± 1.94[b]	49,47[a]	7,06[ab]	27,20[a]

Results (mean values ± S.D.) with different letter are significantly different. E.C. = electric conductivity; O.M. = organic matter content

Table 5. Correlation matrix of the metals and metaloids monitored in soils from Alcalá de Henares

	Al	**As**	**Be**	**Cd**	**Cr**	**Ln Cu**	**Mn**	**Ni**	**Ln Pb**	**Sn**	**Ti**	**Tl**	**V**	**Zn**
Al														
As	**0,706 a**													
	96 **b**													
	0.000 **c**													
Be	**0.642**	0.382												
	97	96												
	0.000	0.001												
Cd	0.498	0.382	0.459											
	91	90	91											
	0.000	0.000	0.000											
Cr	**0.703**	**0.666**	0.355	0.473										
	89	88	89	84										
	0.000	0.000	0.001	0.000										
Ln Cu	**0.708**	0.560	0.360	**0.636**	**0.680**									
	95	94	95	90	88									
	0.000	0.000	0.000	0.000	0.000									
Mn	**0.828**	**0.668**	0.485	0.502	**0.615**	**0.672**								
	95	94	95	89	87	93								
	0.000	0.000	0.000	0.000	0.000	0.000								
Ni	0.103	0.036	0.078	0.040	-0.020	0.153	0.168							
	97	96	97	91	89	95	95							
	0.314	0.730	0.449	0.710	0.856	0.140	0.103							
Ln Pb	**0.609**	0.431	0.307	**0.659**	**0.651**	**0.793**	0.544	0.138						
	96	95	96	90	88	94	94	96						
	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.181						
Sn	0.095	-0.013	0.060	0.227	0.070	0.066	0.018	0.032	0.124					
	93	92	93	88	87	92	91	93	92					
	0.364	0.905	0.569	0.034	0.518	0.533	0.863	0.764	0.238					
Ti	**0.799**	0.548	0.427	0.447	0.552	0.568	0.566	0.048	0.495	0.220				
	95	94	95	89	87	93	93	95	94	91				
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.642	0.000	0.036				
Tl	**0.854**	0.73	**0.819**	0.520	0.578	0.585	**0.753**	0.145	0.469	-0.018	0.566			
	97	96	97	91	89	95	95	97	96	93	95			
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.158	0.000	0.862	0.000			
V	**0.801**	**0.823**	0.525	0.501	**0.713**	0.646	**0.746**	0.133	0.600	-0.027	0.587	**0.815**		
	96	95	96	90	89	94	94	96	95	92	94	96		
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.196	0.000	0.799	0.000	0.000		
Zn	**0.610**	0.571	0.180	**0.639**	0.594	**0.744**	**0.684**	0.139	**0.668**	0.108	0.507	0.478	**0.640**	
	90	89	90	86	85	89	88	90	89	87	88	90	89	
	0.000	0.000	0.090	0.000	0.000	0.000	0.000	0.192	0.000	0.319	0.000	0.000	0.000	

a = Correlation coefficients (r); b = number of samples; c = significance value.

Table 6. Correlation matrix of the variables determined in soils from Alcalá de Henares

	Al	As	Be	Cd	Cr	Ln Cu	Mn	Ni	Ln Pb	Sn	Ti	Tl	V	Zn	E.C.	pH	O.M.	Clay	Silt	Sand
E.C.	0.293 **a**	-0.015	**0.704**	0.235	0.019	0.426	-0.117	-0.350	**0.949**	**0.902**	0.153	0.112	-0.006	-0.202						
	97 **b**	96	97	91	89	95	95	97	96	93	95	97	96	90						
	0.356 **c**	0.964	0.011	0.462	0.953	0.167	0.718	0.265	0.000	0.000	0.635	0.730	0.986	0.530						
pH	0.480	0.108	0.080	0.321	-0.244	0.165	-0.463	-0.157	0.003	0.322	0.535	-0.215	-0.173	-0.118	0.041					
	97	96	97	91	89	95	95	97	96	93	95	97	96	90	97					
	0.115	0.737	0.806	0.308	0.445	0.609	0.130	0.627	0.993	0.308	0.073	0.502	0.591	0.714	0.898					
O.M.	-0.370	0.085	-0.299	-0.007	0.431	-0.054	0.36	0.366	-0.227	-0.438	-0.288	0.168	0.400	0.141	-0.302	**-0.734**				
	97	96	97	91	89	95	95	97	96	93	95	97	96	90	97	97				
	0.237	0.793	0.346	0.982	0.162	0.869	0.250	0.243	0.478	0.154	0.365	0.601	0.197	0.662	0.340	0.007				
Clay	0.440	-0.183	0.577	-0.096	0.479	0.196	0.040	0.200	0.559	0.425	0.211	0.220	0.320	0.232	0.532	-0.105	-0.236			
	97	96	97	91	89	95	95	97	96	93	95	97	96	90	97	97	97			
	0.153	0.568	0.049	0.766	0.115	0.542	0.903	0.533	0.059	0.169	0.510	0.493	0.311	0.468	0.075	0.746	0.461			
Silt	0.527	0.269	0.389	0.135	0.261	**0.720**	-0.397	0.204	0.237	0.249	0.391	-0.032	0.200	-0.129	0.157	0.354	-0.264	0.362		
	97	96	97	91	89	95	95	97	96	93	95	97	96	90	97	97	97	97		
	0.079	0.399	0.211	0.675	0.413	0.008	0.202	0.526	0.458	0.435	0.209	0.922	0.533	0.690	0.625	0.259	0.406	0.247		
Sand	-0.574	-0.137	-0.534	-0.039	-0.378	**-0.646**	0.261	-0.221	-0.387	-0.346	-0.368	-0.078	-0.251	0.030	-0.318	-0.199	0.300	**-0.672**	**-0.929**	
	97	96	97	91	89	95	95	97	96	93	95	97	96	90	97	97	97	97	97	
	0.051	0.672	0.074	0.904	0.229	0.023	0.413	0.490	0.214	0.271	0.239	0.809	0.432	0.927	0.314	0.534	0.344	0.017	0.000	

a = Correlation coefficients (r); b = number of samples; c = significance value.

Table 7. Concentrations of trace elements and significance levels for the elements significantly affected by the area of residence in **children's hair** (6-9 years) in Alcalá de Henares.

Element	**Zone I**	**Zone II**	**Zone IV**	***p***
Cr	0.56 ± 0.10^{a}	0.65 ± 0.15^{b}	0.74 ± 0.14^{c}	<0.001
Hg	0.59 ± 0.36^{a}	1.15 ± 0.99^{b}	1.30 ± 0.92^{b}	<0.01

Concentration values [arithmetic mean (μg/g) ± SD] with different letter are significantly different.

Table 8. Concentrations of trace elements and significance levels for the elements significantly affected by the area of residence in **adolescents' hair** (13-16 years) in Alcalá de Henares.

Element	Zone I	Zone II	Zone III	Zone IV	*p*
Cr	0.54 ± 0.13^{a}	0.54 ± 0.12^{a}	0.43 ± 0.12^{b}	0.44 ± 0.13^{b}	<0.01
Cu	9.40 ± 4.30^{a}	14.55 ± 8.46^{b}	13.58 ± 7.22^{b}	12.37 ± 7.61^{ab}	<0.05
Hg	0.45 ± 0.28^{a}	0.45 ± 0.29^{a}	0.54 ± 0.32^{a}	0.89 ± 0.63^{b}	<0.05
Pb	0.57 ± 0.38^{a}	1.13 ± 0.71^{b}	0.56 ± 0.39^{a}	0.72 ± 0.46^{a}	<0.01
Sn	1.67 ± 0.52^{a}	1.52 ± 0.48^{ab}	1.53 ± 0.60^{a}	1.20 ± 0.39^{b}	<0.05

Concentration values [arithmetic mean (μg/g) ± SD] with different letter are significantly different.

Table 9. Concentrations of metals and metalloids (μg/g) in hair **of children and adolescents** in Alcalá de Henares, Spain.

Element	LoD	Children	Adolescents	*p*
Al	0.2	9.05 ± 7.68	5.34 ± 2.96	<0.01
As	0.02	ND	ND	-
Be	0.05	ND	ND	-
Cd	0.005	0.52 ± 0.24	0.11 ± 0.14	<0.001
Cr	0.01	0.66 ± 0.15	0.50 ± 0.13	<0.001
Cu	0.01	19.24 ± 26.02	11.99 ± 6.85	<0.05
Hg	0.01	1.10 ± 0.91	0.55 ± 0.40	<0.001
Mn	0.01	0.30 ± 0.20	0.14 ± 0.08	<0.001
Pb	0.01	1.48 ± 1.29	0.70 ± 0.52	<0.001
Sn	0.01	1.29 ± 0.52	1.52 ± 0.53	<0.01
Ti	0.02	0.88 ± 0.60	0.87 ± 0.19	NS
Tl	0.01	ND	ND	-
Zn	0.02	85.58 ± 47.06	148.25 ± 25.6	<0.001

LoD = limit of detection. Results are presented as mean values ± S.D. (in μg/g); NS = Differences are not statistically significant ($p > 0.05$); ND = Not detected.

Figure 1. Study area and sampling sites.

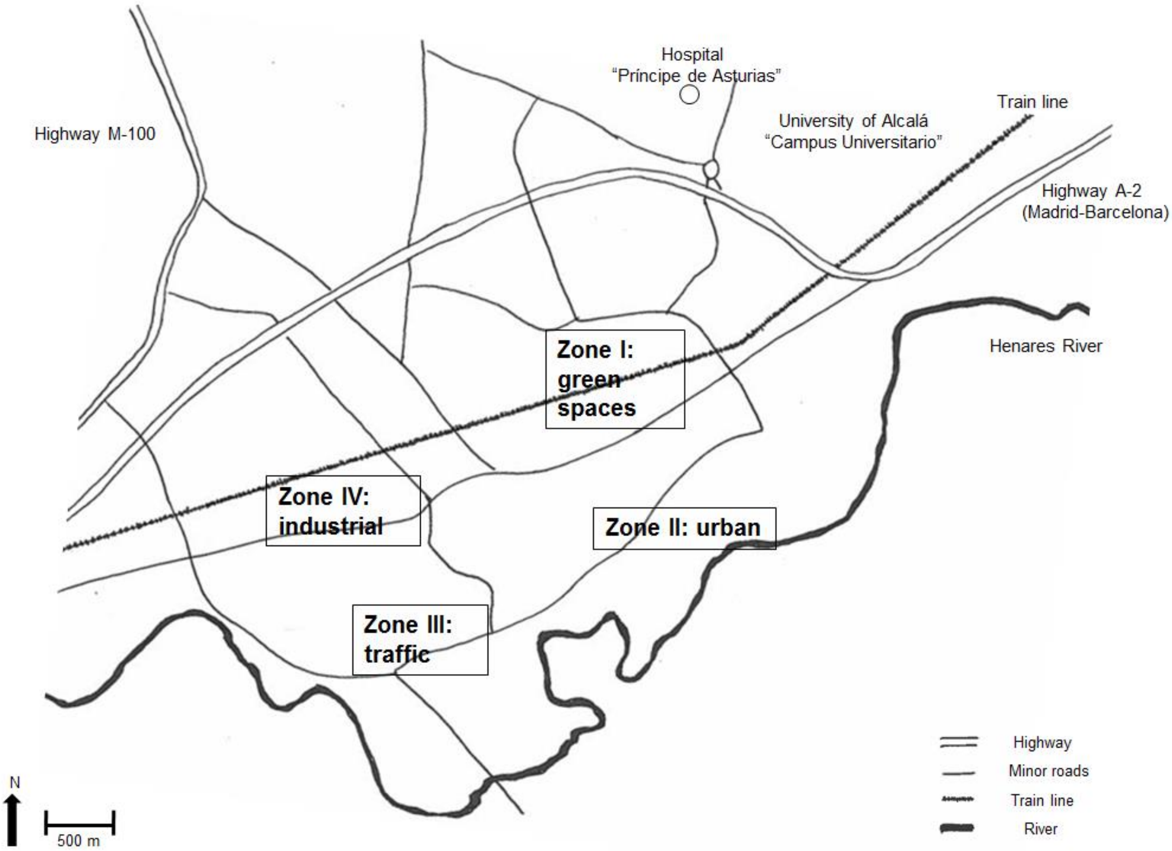
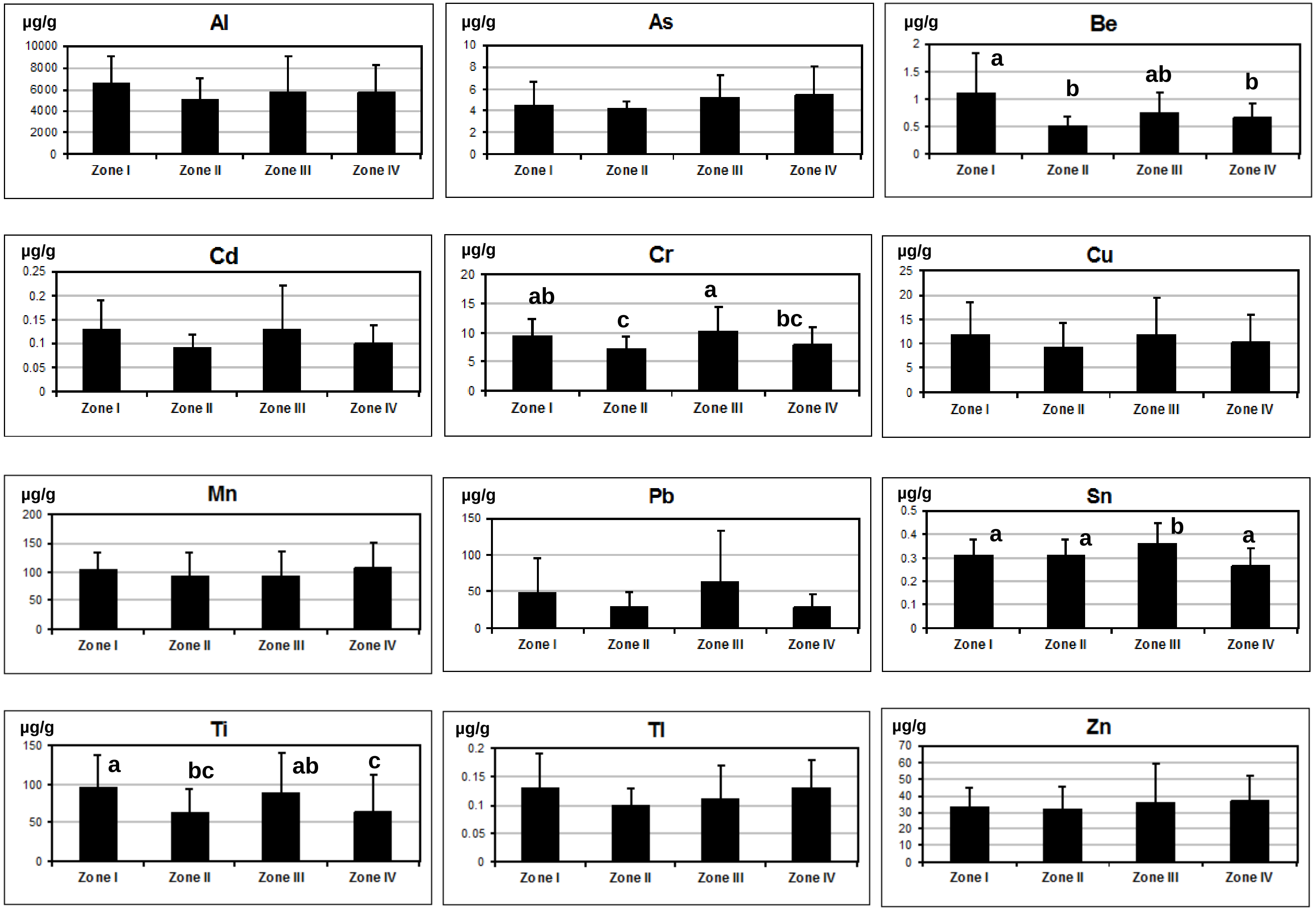
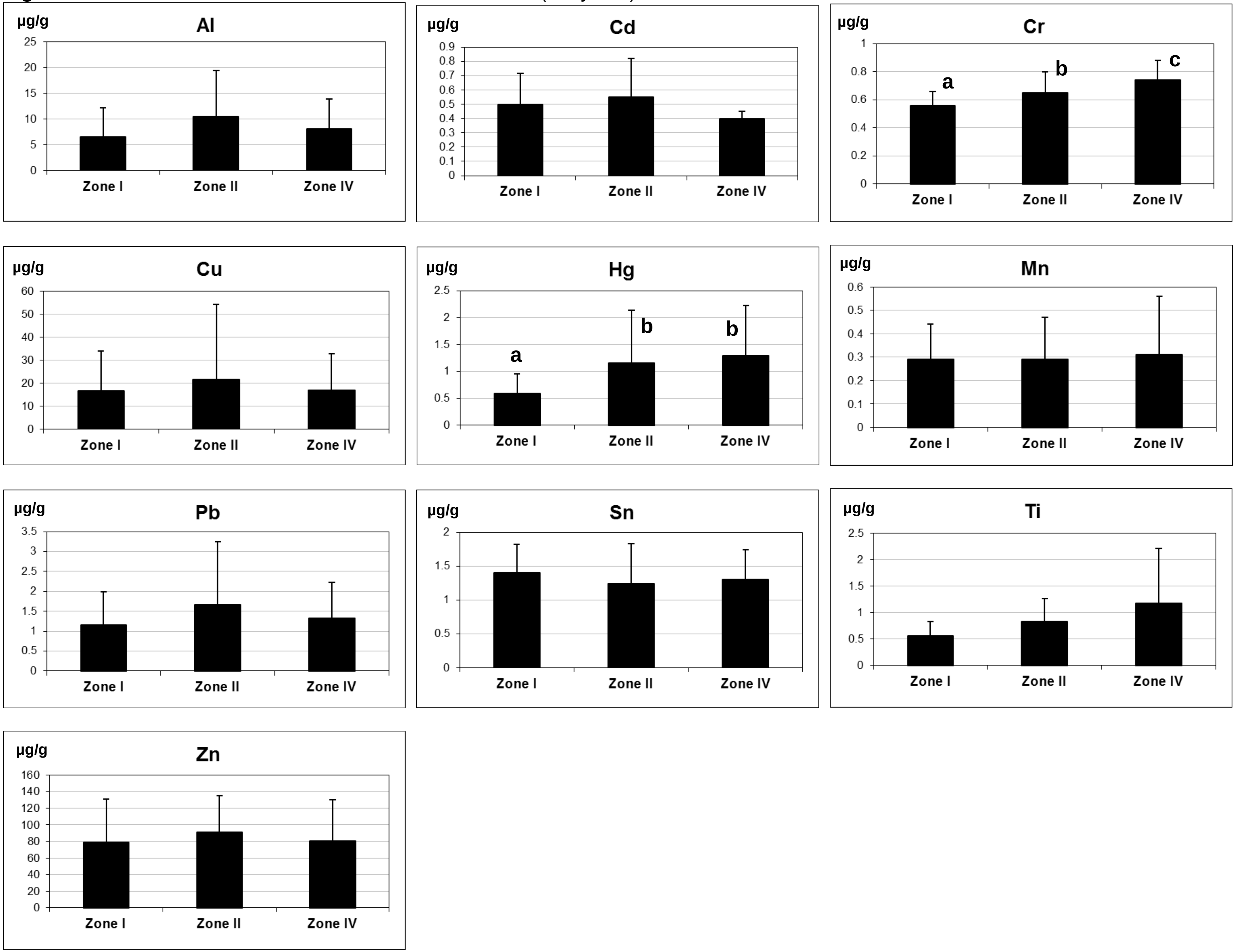


Figure 2. Levels of metals and metalloids in urban topsoils monitored in each zone in Alcalá de Henares.



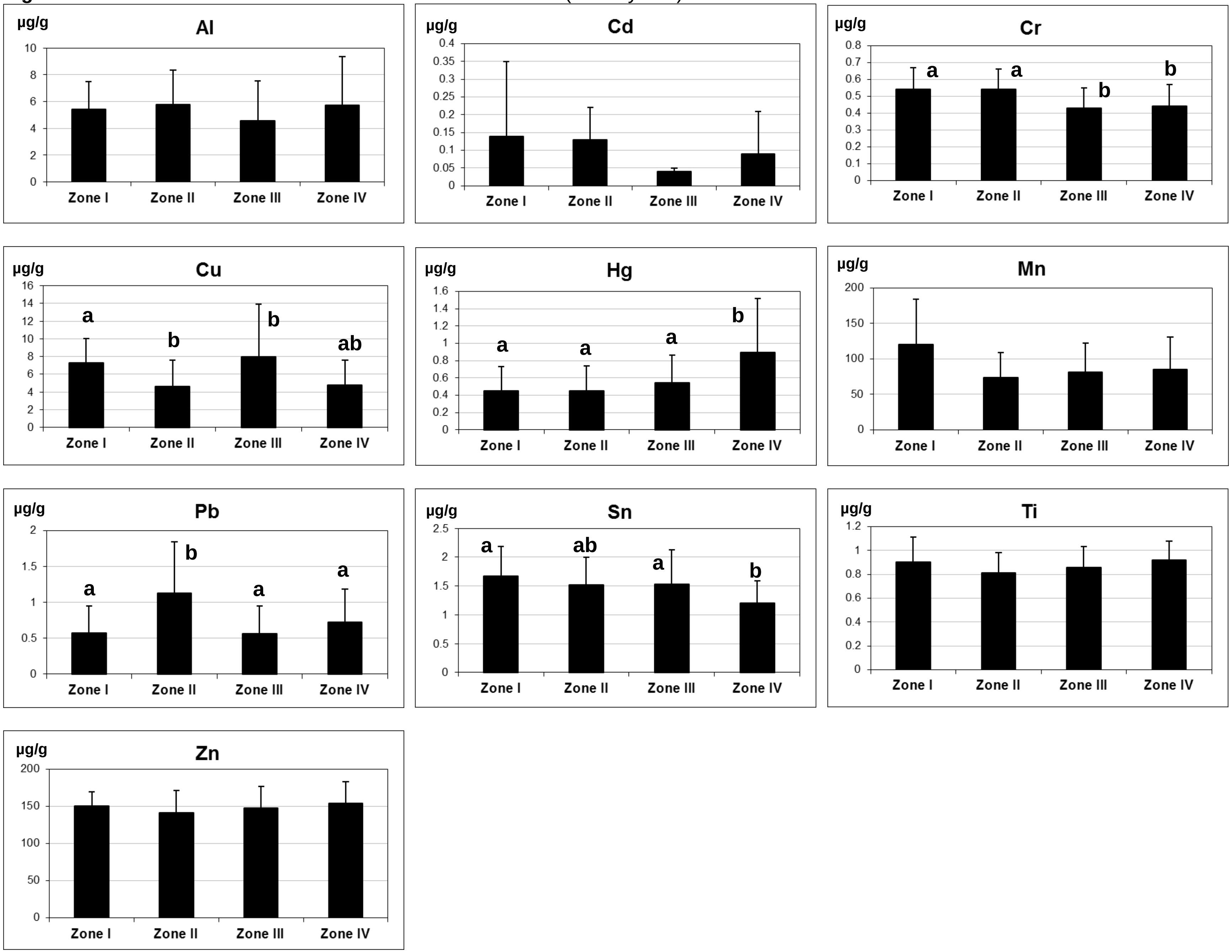
Concentration values (arithmetic mean (µg/g) ± SD) with different letter are significantly different.

Figure 3. Levels of metals and metalloids in **children's hair** (6-9 years) in each zone monitored in Alcalá de Henares.



Concentration values (arithmetic mean (µg/g) ± SD) with different letter are significantly different.

Figure 4. Levels of metals and metalloids in **adolescents' hair** (13-16 years) in each zone monitored in Alcalá de Henares.



Concentration values (arithmetic mean (μg/g) ± SD) with different letter are significantly different.